

## ULTRASTRUCTURAL DIFFERENCES IN THE EGGS AND OVARIAN FOLLICLE CELLS OF *CAPITELLA* (POLYCHAETA) SIBLING SPECIES

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### ABSTRACT

Ultrastructural studies of ovarian follicle cells and mature eggs in four sibling species in the polychaete genus *Capitella* have revealed distinct and consistent morphological differences that parallel in some respects the differences between the species in egg size, and embryonic and larval development. *Capitella* spp. I and II are extremely similar in all respects: the follicle cells lack lipid and contain a modest amount of glycogen; the mature eggs are rich in lipid and glycogen and contain very similar proteid yolk granules. In both species mature eggs have a characteristic electron-dense band and a zone of mitochondria in the cortical ooplasm. These sympatric species have eggs that are similar in size and lecithotrophic larvae that are planktonic for only a short time. *Capitella* sp. III (*Capitella jonesi*) has ovarian follicle cells containing a small amount of lipid and no glycogen, while the mature eggs have a small amount of lipid, abundant glycogen, and large proteid yolk granules. These small eggs show no evidence of an electron-dense band or any concentration of mitochondria in the cortical ooplasm. This species has planktotrophic larvae that remain in the plankton for many weeks. *Capitella* sp. IIIa has ovarian follicle cells rich in both lipid and glycogen. The large mature eggs are rich in lipid, have relatively little glycogen, and have abundant proteid yolk granules. The cortical ooplasm contains electron-dense material similar to that observed in the eggs of species I and II but it is distributed in a discontinuous band. This species has direct development, and juvenile worms emerge from the parental brood tube after metamorphosis. The egg envelopes and microvilli of the eggs of all four sibling species undergo substantial morphological changes following release from the ovary into the coelom.

The significance of these morphological and biochemical differences between the species is not known, but the lack of intraspecific variation in these characters suggests that their presence or absence reflects specific differences in the processes of yolk formation and utilization.

### INTRODUCTION

Comparative studies of metazoan sperm structure have demonstrated considerable interspecific variation unprecedented in other cell types. Since one of the events in speciation is the creation of barriers to crosses between new species and the parental forms, it is generally thought that the modifications in sperm morphology and their properties may contribute to the establishment of such a barrier. Baccetti and Afzelius (1976) point out that species specificity not only resides in the genetic material bound in the nucleus of the spermatozoan but is also imprinted in the morphology of the cell itself. Thus in some nereid polychaetes, for example,

we observe markedly different sperm types in morphologically similar species (Hauenschild, 1951; Durchon, 1955). Comparative studies of egg morphology are rare however, because at the light microscope level at least, female germ cells show far less structural variation. Aside from differences in volume, color, general shape, or perhaps features of the egg envelope, there are fewer morphological parameters available for cytological comparisons than in sperm. However, comparative light microscope observations on egg morphology in closely related polychaete species have been reported in orbiniids (Anderson, 1961) spionids, (Blake, 1969) and cirratulids (Gibbs, 1971).

It seems reasonable to assume that in some cases, barriers to cross fertilization between incipient species might be reflected by morphological changes in the eggs as they are in sperm. Recent comparative ultrastructural studies of oogenesis in four species of the sibling complex of *Capitella* have revealed distinct and consistent morphological differences in the ovarian follicle cells and mature eggs among members of this group. The differences include variation in the relative quantities of nutritive materials stored in the mature egg which in turn may reflect differences in the energetic requirements of the larvae. These findings are the first to our knowledge, to describe ultrastructural differences in the female germ cells of closely related invertebrate species.

*Capitella capitata* (Fabricius), formerly regarded as an opportunistic, cosmopolitan polychaete species characteristically present in dense populations in highly disturbed environments (Grassle and Grassle, 1974; Pearson and Rosenberg, 1978), recently has been shown to be a complex of more than ten sibling species (Grassle and Grassle, 1976; Grassle, 1980). Although the morphologies of the adults are very similar, the species show striking differences in life history features including reproductive mode, breeding season, egg size, and dispersal capability of the larvae. Marked differences are also observed in the electrophoretic mobilities of allozymes at selected enzyme loci, indicating that genetic distances between species are great. In addition, individuals of the various sibling species do not hybridize in the laboratory or in the field (Grassle and Grassle, 1976; Grassle, 1980). The *Capitella* species complex is particularly interesting because it represents a wide range of reproductive variation from species I, which has large eggs (260  $\mu\text{m}$ ), small broods (30–400 eggs), and a lecithotrophic larval dispersal phase of only a few hours to species III (*Capitella jonesi*) which has small eggs (50  $\mu\text{m}$ ), large broods (200–1000 eggs), and a planktotrophic larval phase of five weeks or more. The length of oogenesis also varies from 5–7 days in species I to 40–50 days in species IIIa. Breeding seasons range from a short period in winter or early spring (species Ia and III) to those which breed throughout the year (species I and II).

#### MATERIALS AND METHODS

Animals used in this study belong to four genetically distinct sympatric *Capitella* species collected from the field in the vicinity of Woods Hole, Massachusetts. The material from *Capitella* spp. I, II, and IIIa was obtained from laboratory strains. *Capitella jonesi* (*Capitella* sp. III, Grassle and Grassle, 1976) individuals were collected in the field and maintained in the laboratory. Worms were kept in filtered, standing sea water at 15°C and were provided with azoic mud as food and substrate. Food and water were changed at bi-weekly intervals. For electron microscopy, genital segments from females and hermaphrodite individuals at various stages of sexual maturity were cut into small pieces. Tissue fixation and preparation were according to procedures previously outlined in Eckelbarger (1979). Sections of embedded tissue

were cut on a Porter-Blum MT-2B ultramicrotome with a diamond knife, stained with aqueous saturated uranyl acetate followed by lead citrate, and examined with a Zeiss EM-9S2 electron microscope.

## RESULTS

The ovaries of all members of the *Capitella* sibling species complex examined are paired, sac-like organs, suspended by mesenteries in the ventral coelomic cavity throughout the mid-body segments. Each ovary consists of a sac (or follicle) formed by somatic follicle cells in which the oocytes complete vitellogenesis. The follicle cells are modified coelomic peritoneal cells which become hypertrophic prior to vitellogenesis and undergo marked cytological changes including the development of extensive arrays of rough endoplasmic reticulum (RER) and numerous Golgi complexes (Fig. 1). In the medial region of the ovary, developing oocytes remain in intimate contact with the layer of follicle cells but gradually lose the association as they reach their maximum size and expand into the lateral region of the ovary where they cease growth and await ovulation. When release from the ovary occurs, possibly resulting from the active migration of follicle cells from the surface of the eggs (Eckelbarger and Grassle, 1982), the eggs enter the coelom where they float freely for a variable period before being spawned by the female. Laboratory observations indicate that the period of coelomic egg storage in the female is minimal when a sexually mature male is present in the culture. Most ultrastructural features of the eggs in the lateral region of the ovary are indistinguishable from those floating freely in the coelom, although the egg envelopes of all four sibling species and the cortical ooplasm in the egg of species IIIa undergo additional differentiation following ovulation. All ovulated eggs have a prominent germinal vesicle and there is no indication that further maturation occurs before spawning. Numerous ovarian follicle cells, ovarian eggs, and ovulated eggs from many individuals were carefully examined ultrastructurally in all stages of vitellogenesis in the four sibling species of *Capitella*. No intraspecific variation in follicle cell and mature egg morphology was apparent.

### *Follicle cells*

The ovarian follicle cells of these four members of the *Capitella* sibling species complex have many similar ultrastructural features. These include the presence of large nuclei each with a prominent nucleolus, extensive RER, Golgi complexes, a variety of membrane-bound, heteromorphic electron-dense bodies resembling lysosomes, bundles of fibrils measuring 5–7 nm, mitochondria, and often a pair of centrioles (Fig. 2). However, there are consistent differences in the relative number of glycogen granules and lipid droplets found in these cells throughout the life history of each species (Table I). Species I and II follicle cells are similar in not possessing lipid droplets at any stage of oogenesis (Figs. 2, 4) whereas species IIIa cells have an abundant quantity (Fig. 3). The follicle cells of species III have a small number of lipid droplets. Except for species III, the follicle cells of each of the species contain glycogen (Figs. 3, 4). These differences are readily apparent after observing semi-serial sections from numerous ovaries in many individuals in different stages of sexual maturity. Since quantitative methods of comparison between the follicle cells of various siblings would be difficult, we have made qualitative, ultrastructural comparisons based on the absence of lipid or glycogen or its presence in small, moderate, or abundant quantities (Table I).

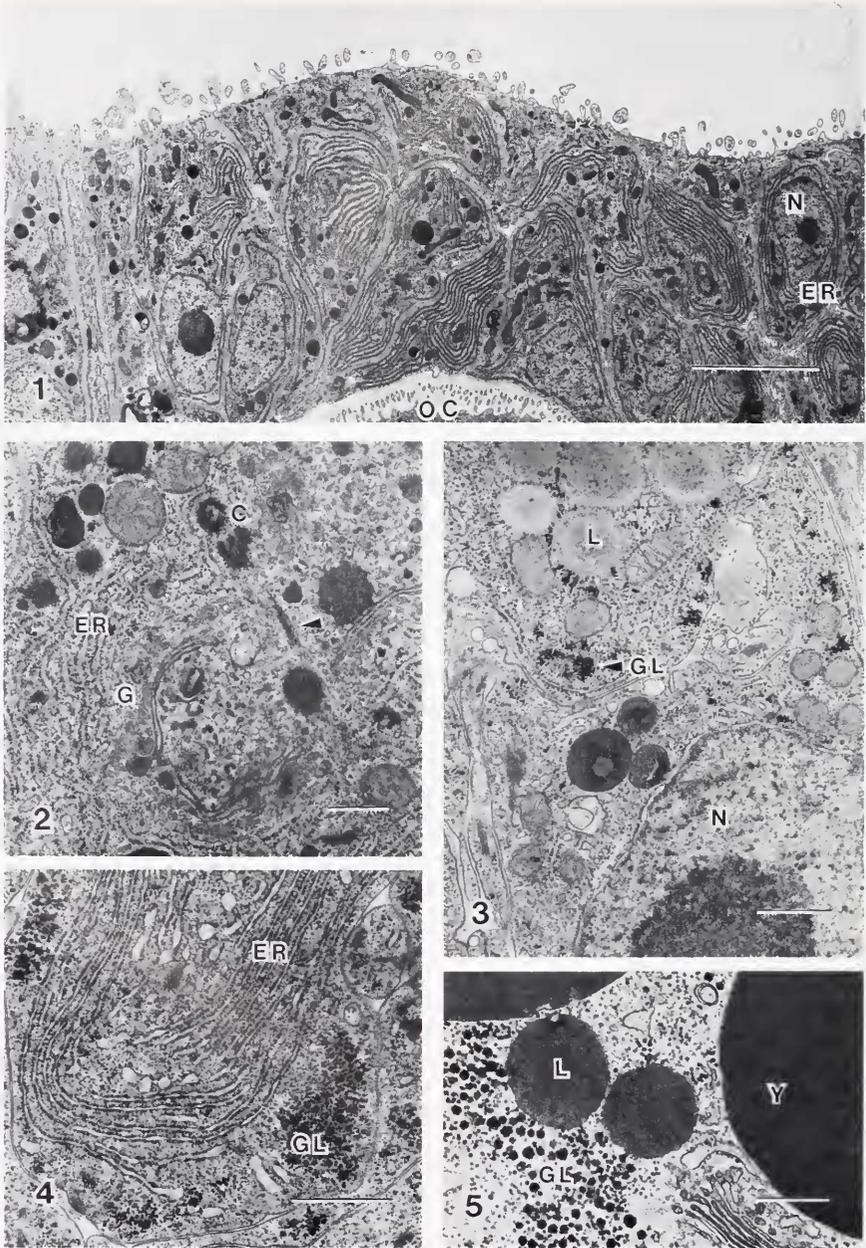


FIGURE 1. Stratified layer of follicle cells composing the wall of the ovary in *Capitella* species III. N, nucleus; ER, rough endoplasmic reticulum; OC, vitellogenic oocyte. Bar = 5  $\mu$ m.

FIGURE 2. Follicle cell from ovary of *Capitella* species I showing centrioles (C), Golgi complex (G), fibrils (arrowhead) and rough ER. Bar = 0.6  $\mu$ m.

FIGURE 3. Lipid (L) droplets in follicle cell of *Capitella* IIIa ovary. N, nucleus; GL, glycogen. Bar = 1.5  $\mu$ m.

FIGURE 4. Rough ER and electron-dense glycogen granules (GL) in the follicle cells of *Capitella* species II. Bar = 2  $\mu$ m.

FIGURE 5. Large membrane-bound proteid yolk (Y), small lipid droplets (L) and glycogen granules (GL) in the mature egg of *Capitella* species III. Bar = 1.5  $\mu$ m.

TABLE I

*Ultrastructural features of eggs and follicle cells of Capitella sibling species*

	Egg diameter ( $\mu\text{m}$ )	Larvae in plankton	Follicle cells	Egg	Yolk granule diameter ( $\mu\text{m}$ )*	Egg envelope (coelomic eggs)	Cortical ooplasm (coelomic eggs)
SPECIES I	260 by 180	Several hours	Lipid <sup>-</sup> Glycogen <sup>++</sup>	Lipid <sup>++</sup> Glycogen <sup>+++</sup>	3.1	Envelope 1.2 $\mu\text{m}$ thick Slender microvilli with smooth surfaces	Electron-dense band Mitochondria concentrated in band
SPECIES II	190	Several days	Lipid <sup>-</sup> Glycogen <sup>+</sup>	Lipid <sup>++</sup> Glycogen <sup>+++</sup>	3.1	Envelope 0.8 $\mu\text{m}$ thick Smooth microvilli	Electron-dense band Mitochondria concentrated in band
SPECIES III	50	Approx. 5 weeks	Lipid <sup>+</sup> Glycogen <sup>-</sup>	Lipid <sup>+</sup> Glycogen <sup>+++</sup>	4.75	Envelope 0.75 $\mu\text{m}$ thick Microvilli granulated on lateral surfaces	No electron-dense material or mitochondrial band Random distribution of organelles
SPECIES IIIA	250	None	Lipid <sup>+++</sup> Glycogen <sup>++</sup>	Lipid <sup>+++</sup> Glycogen <sup>+</sup>	3.7	Envelope 0.6 $\mu\text{m}$ thick Short, stubby microvilli with granular tips	Intermittent band of electron-dense material Random distribution of organelles.

- none

+ rare

++ moderate

+++ abundant

\* based on the average of 100 of the largest yolk granules

*Eggs*

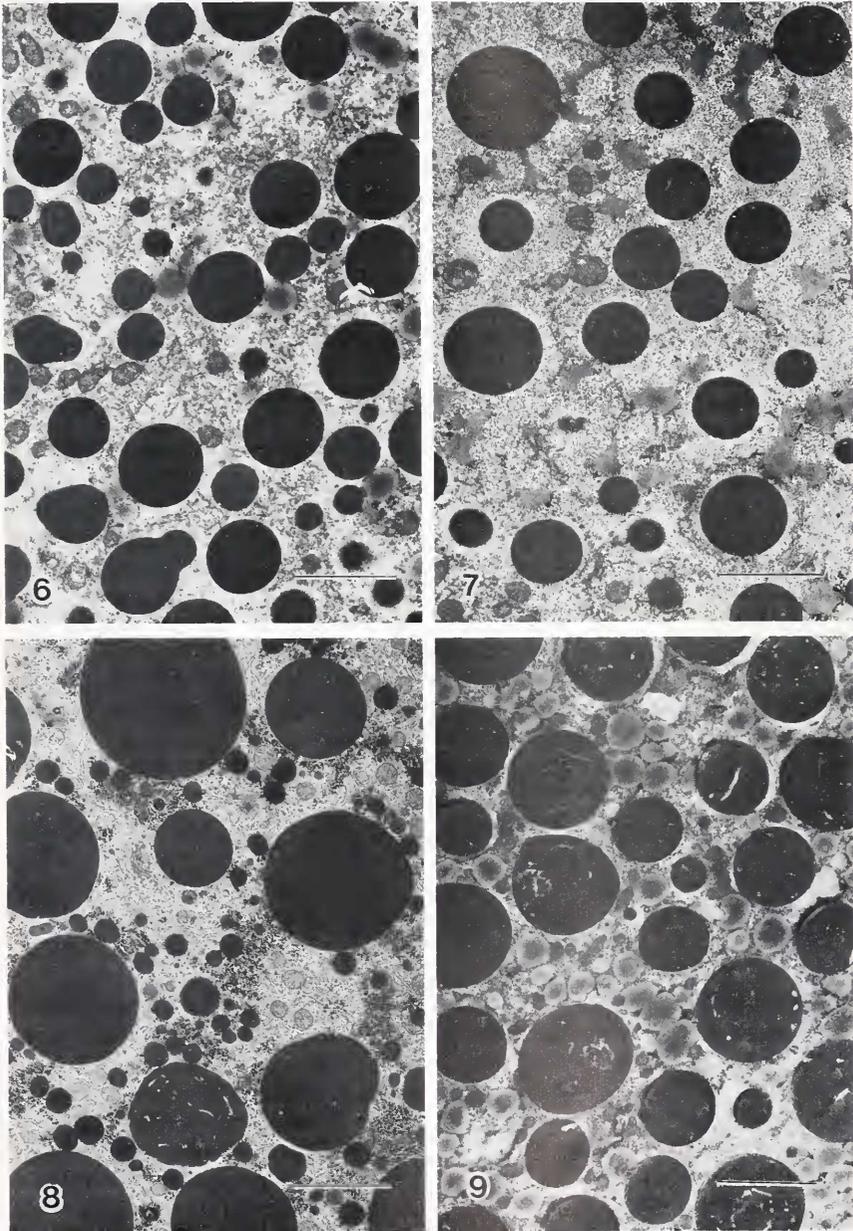
The mature eggs of all four sibling species are creamy-white to pale yellow in color. As many as three types of nutritive material or yolk are recognizable in the eggs including large, membrane-bound, spherical, proteid yolk bodies, small unbound lipid droplets, and electron-dense glycogen granules (Fig. 5). The formation of these yolk materials has been described in a previous publication (Eckelbarger and Grassle, 1982). The proteid yolk granules are usually spherical in shape and vary in size within the same egg. This variation probably results, in part, from a sectioning artifact in which only a portion of some granules are visible in any one section. The maximum diameter of yolk granules does show considerable interspecific variation (based on measurement of 100 of the largest yolk granules per egg). The smallest (averaging  $3.1 \mu\text{m}$ ) is found in species I and II, and the largest (averaging  $4.75 \mu\text{m}$ ) in species III. Those of species IIIa are intermediate in size, averaging  $3.7 \mu\text{m}$ . Qualitative observations suggest that the number of these granules per unit area is approximately the same in the ovulated egg of all four sibling species (Figs. 6–9) with the exception of species IIIa which appears to have many more (Fig. 9).

In addition to differences in species-specific egg diameter and yolk granule diameter, there are also differences in the arrangement and location of cortical organelles in the eggs. These differences are first observed in the early to middle stages of vitellogenesis and persist in some species even after release from the ovary. The cortical regions of the eggs of species I and II are free of all organelles except for a distinct band of mitochondria (Figs. 10, 11). Apart from their concentration in a cortical monolayer, these mitochondria are indistinguishable from those present in the remainder of the ooplasm. There is also a thin (100–120 nm) layer of amorphous electron-dense material parallel to the oolemma (Figs. 10, 12, 15, 16, 19). This circumferentially arrayed band sometimes appears, in favorable sections, to consist of densely packed but randomly oriented filaments which extend into the adjacent microvilli in some instances. This band appears during early vitellogenesis (Fig. 12) while the mitochondrial band appears during the middle stages of yolk formation. The eggs of species III and IIIa lack the monolayer of mitochondria. The electron-dense band is absent from the cortical ooplasm of species III eggs but is present as a discontinuous band in the eggs of species IIIa. The cortical region of the egg of species III contains the same random mixture of yolk granules and mitochondria as the remainder of the egg (Fig. 13) at all stages of development while that of species IIIa possesses a unique organelle-free cortical zone up to  $2.6 \mu\text{m}$  wide (Fig. 14) which persists until ovulation. Following release from the ovary, the organelle-free zone disappears and the ooplasmic components become evenly distributed (Fig. 18). The post-ovulatory arrangement of ooplasmic organelles remains unchanged in the eggs of species I, II, and III (Figs. 15–17).

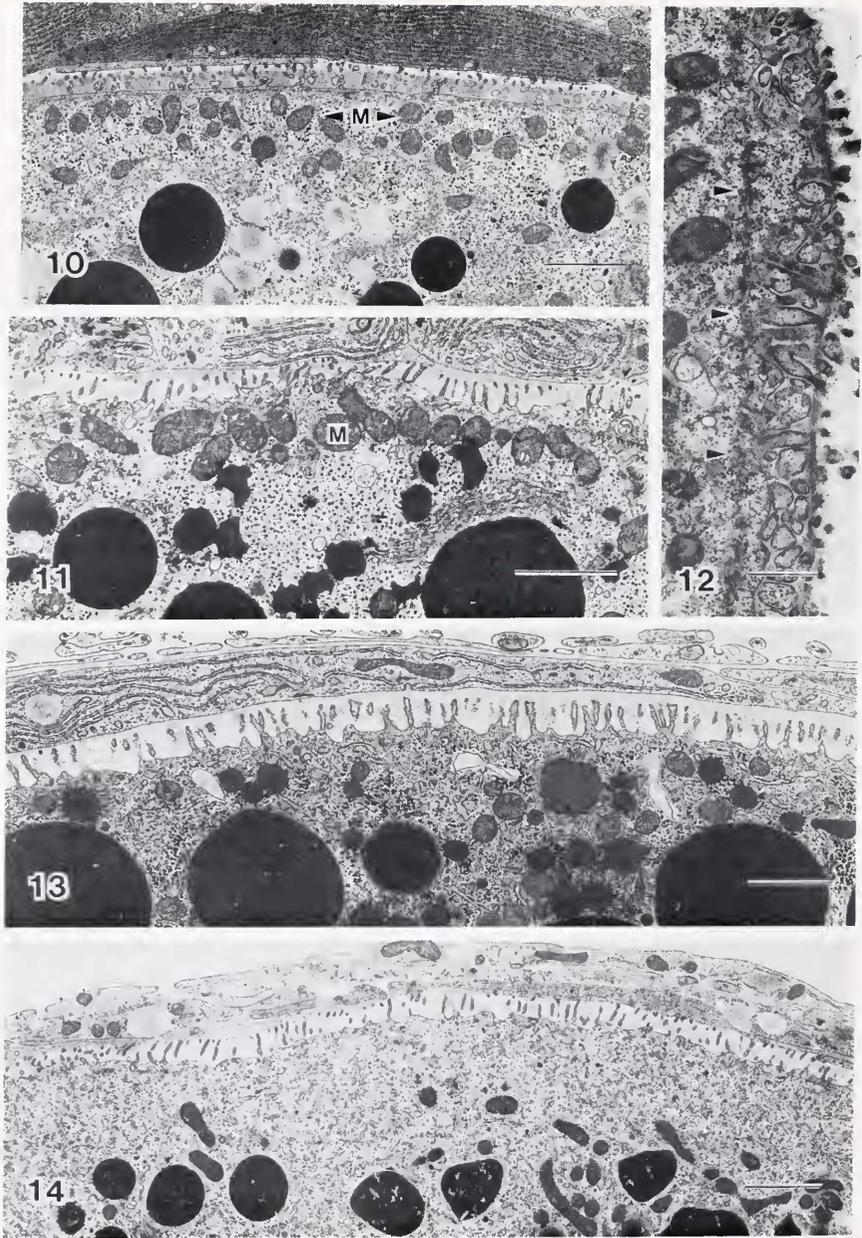
Prior to release of the eggs into the coelom, the egg envelopes of all four species are similar in thickness and in having short, branching microvilli and a simple electron-dense layer extending from the oolemma to near the tips of the microvilli (Figs. 19–22). Following ovulation, however, substantial changes are observed in the egg envelope and the morphology of the egg microvilli (Figs. 23–26). The envelope varies in thickness from  $1.2 \mu\text{m}$  in species I to  $0.6 \mu\text{m}$  in species IIIa. The microvilli covering the eggs of species I, II, and III have flattened, swollen, or branching tips (Figs. 23–25), while those of species IIIa are short with constricted tips bearing small granules (Fig. 26). The lateral surfaces of the microvilli in species I, II, and IIIa are relatively smooth while those of species III have a granulated appearance (Fig. 25). Table I summarizes the ultrastructural differences between the coelomic eggs and ovarian follicle cells of the four sibling species.

## DISCUSSION

The occurrence of sibling species in polychaetes in which members of natural populations are morphologically similar or identical yet reproductively isolated has been revealed through the analysis of morphological data, reproductive processes,



FIGURES 6-9. Yolk bodies from mature (coelomic) eggs of *Capitella* sibling species. Figure 6, species I; Figure 7, species II; Figure 8, species III; Figure 9, species IIIa. Bars = 3.0  $\mu$ m.



FIGURES 10, 11, 13, 14. Cortical ooplasm of eggs in lateral region of ovary. Eggs have completed growth and vitellogenesis.

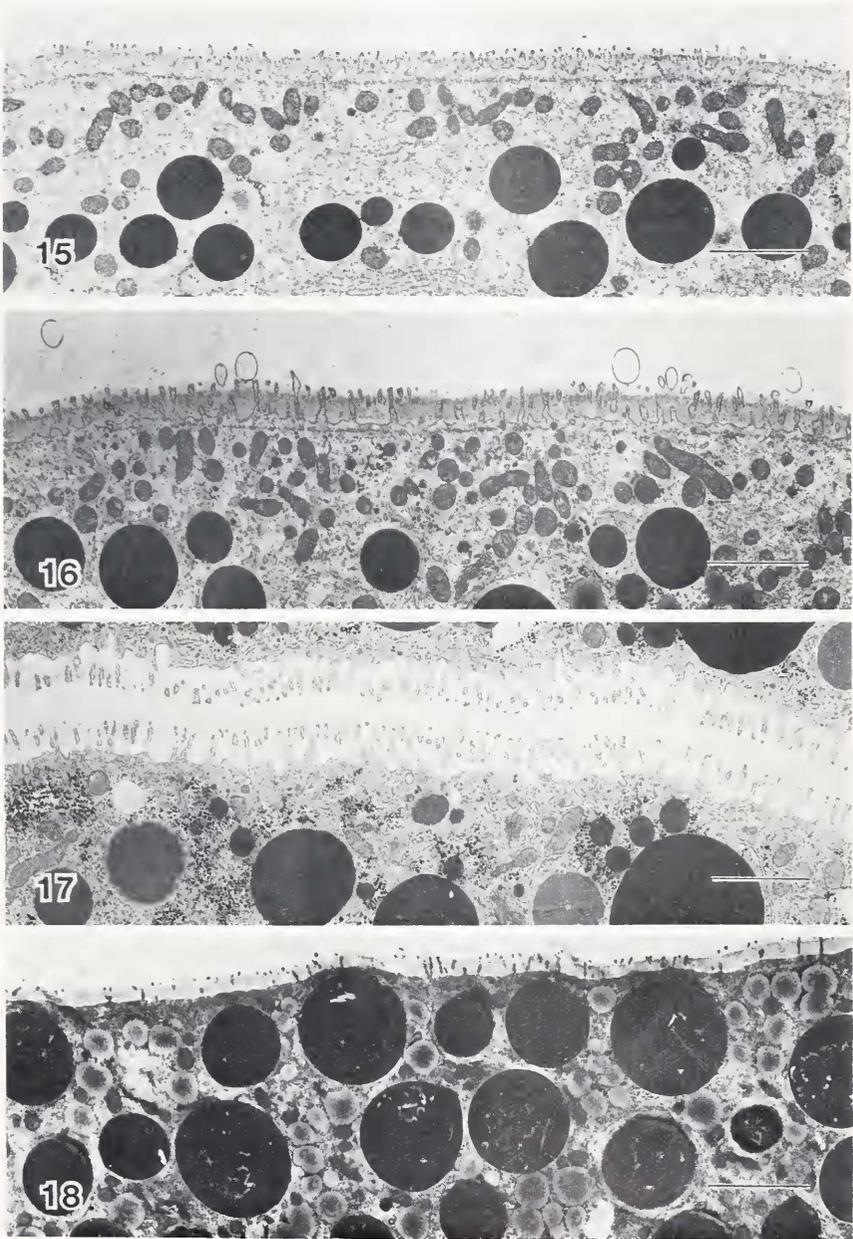
FIGURE 10. Cortical ooplasm of *Capitella* species I showing band of mitochondria (M). Bar = 2  $\mu\text{m}$ .

FIGURE 11. Cortical ooplasm of *Capitella* species II showing band of mitochondria (M). The thin layer of amorphous electron-dense material parallel to the oolemma is seen to the right of the mitochondria in this section.

FIGURE 12. Band of amorphous material (arrowheads) adjacent to newly forming microvilli in cortical ooplasm of early vitellogenic oocyte of *Capitella* species I. Bar = 0.53  $\mu\text{m}$ .

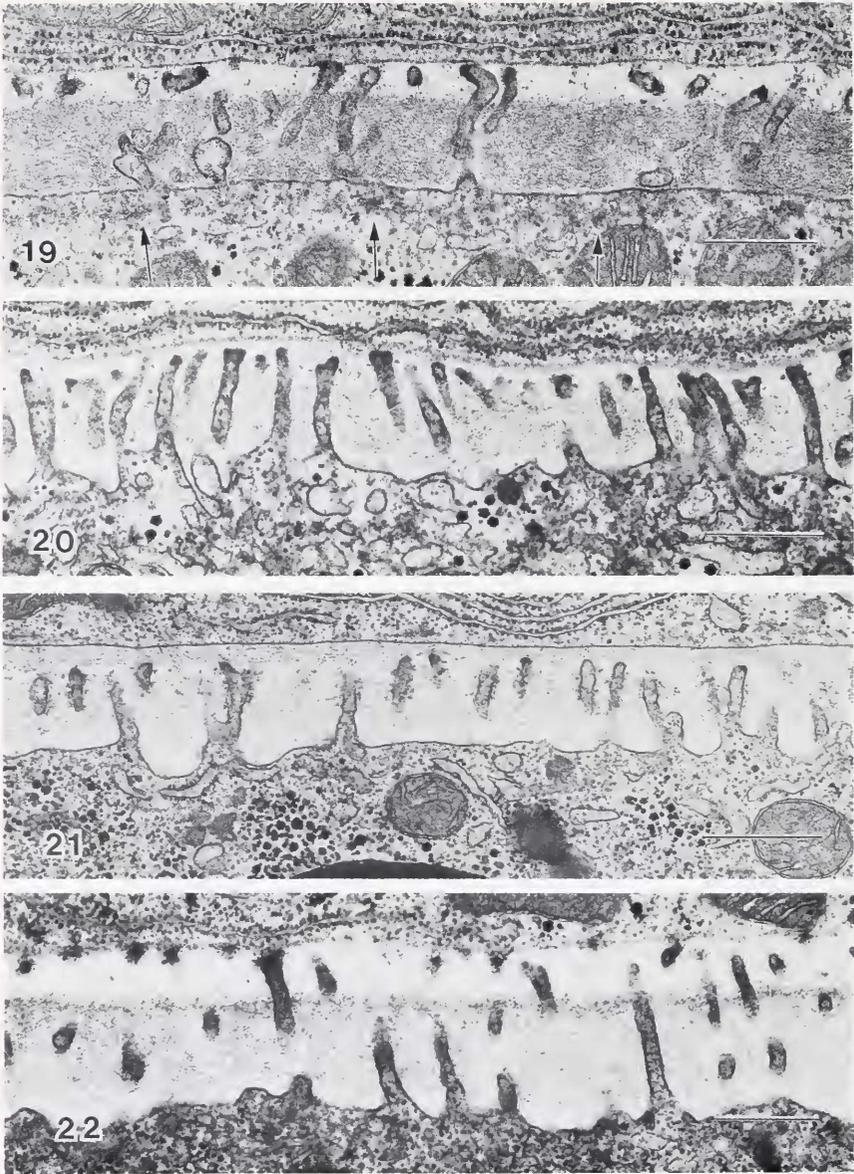
FIGURE 13. Cortical ooplasm of *Capitella* species III egg. Bar = 1.3  $\mu\text{m}$ .

FIGURE 14. Cortical ooplasm of *Capitella* species IIIa showing organelle-free zone. Bar = 1.8  $\mu\text{m}$ .



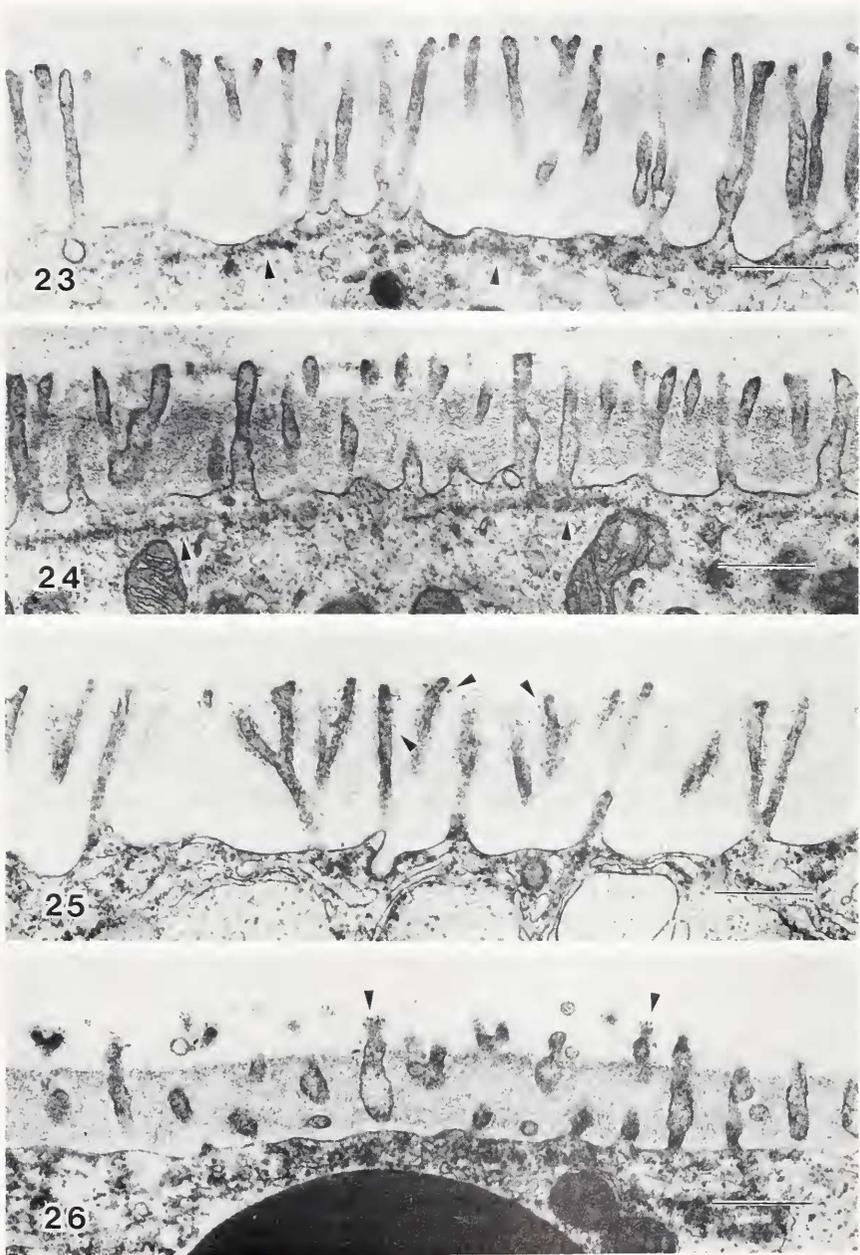
FIGURES 15-18. Cortical ooplasm of coelomic eggs of *Capitella* sibling species. Figure 15, species I; Figure 16, species II; Figure 17, species III; Figure 18, species IIIa. Bars = 3.0  $\mu\text{m}$ .

physiological responses, and electrophoretic patterns of related enzymes (see review by Rice and Simon, 1980). The present paper is the first to our knowledge to describe interspecific differences in the eggs and follicle cells of sibling species in any invertebrate. These findings are especially interesting in that not only is interspecific



FIGURES 19–22. Egg envelopes of ovarian eggs of *Capitella* sibling species. Figure 19, species I; Figure 20, species II; Figure 21, species III; Figure 22, species IIIa. Note the amorphous material (arrows) parallel to the oolemma in Figure 19. Bars = 0.63  $\mu\text{m}$ .

variation on the ultrastructural level demonstrated but also that the variation occurs in the female gamete which generally shows little gross morphological variation. The significance of differences in cortical organelle distribution or type of nutrient material in the eggs of *Capitella* sibling species is not readily apparent but it does not appear to bear any obvious relationship to egg size, cleavage pattern, or type of larval development.



FIGURES 23–26. Egg envelopes of coelomic eggs of *Capitella* sibling species. Figure 23, species I; Figure 24, species II; Figure 25, species III; Figure 26, species IIIa. Note the band of amorphous material (arrowheads) adjacent to the oolemma in Figures 23 and 24. Note also the granules attached to the lateral surfaces of the microvilli in Figure 25 and to the microvillar tips in Figure 26. Bars = 0.6  $\mu$ m.

The use of ultrastructural characters in phylogeny and systematics is gradually gaining support (see review by Tyler, 1979). With regard to *Capitella*, some of the ultrastructural differences observed in the eggs of the four sibling species are further

evidence of morphological divergence in this taxonomically difficult species group and may have systematic applications. Some features of the eggs such as the cortical mitochondria in species I and II are not strictly ultrastructural characters since they are discernible with careful light microscopy. However, the cortical band of amorphous material observed in the eggs of species I, II, and IIIa, is only visible with electron microscopy. These additional morphological features may be of systematic importance when combined with the abundant information already available on adult morphology, genetics, and reproductive and life history characteristics (Grassle and Grassle, 1976; Grassle, 1980). Interspecific differences in the relative size, number, and morphological features of the large proteid yolk granules also appear to exist, although it is difficult to establish homology between them. Although they appear to have similar origins (Eckelbarger and Grassle, 1982), it is likely they have very different chemical composition despite their morphological similarity. The use of various morphological features of yolk granules as systematic characters has been proposed in some invertebrate oocytes (Gremigni, 1979) although this approach has been strongly criticized (Tyler, 1981).

The concentration of mitochondria in the cortical ooplasm in the eggs of *Capitella* species I and II is unusual for a polychaete but not uncommon for other animal oocytes (Raven, 1961; Arnold, 1971; Boyer 1972). Localization or stratification of ooplasmic components was termed "ooplasmic segregation" by Costello (1948) and quite often is restricted to the animal pole or polar lobe of the egg (Allen, 1961; Raven, 1961; Anderson and Huebner, 1968; Huebner and Anderson, 1976). In *Diopatra cuprea*, for example, Anderson and Huebner (1968) found yolk granules to be vegetally located and lipid and mitochondria were found in the animal pole. This localized stratification was even retained during early cleavage. Costello (1945, 1948) reported ooplasmic segregation in *Nereis limbata* only following fertilization. Recently, Eckberg (1981), using electron microscopy to study the eggs of *Chaetopterus pergamentaceus*, reported that cytoplasmic components are localized in different regions of the egg and that this localization is maintained as the embryo undergoes cleavage and differentiation. Hess (1971) noted that ooplasmic organelles such as yolk bodies, mitochondria, and endoplasmic reticulum, as well as cellular products such as various types of RNA and metabolites, are unevenly distributed during ooplasmic segregation but are later evenly distributed to the blastomeres during the process of cleavage. The presence of a cortical, organelle-free zone in the egg of species IIIa prior to ovulation, and its disappearance following release from the ovary, is a developmental event previously unreported in an annelid egg. Its significance is unknown.

The functional importance of mitochondrial segregation in *Capitella* eggs is unknown. It is clearly tempting to try to relate ooplasmic segregation to mosaic egg development. However, it has been demonstrated that displacement of cell organelles by reverse centrifugation of some mosaic eggs does nothing to alter development (Clement, 1968). Huebner and Anderson (1976) suggested that a similar distribution of cortical mitochondria in the egg of the hemipteran *Rhodnius prolixus* might reflect the need for an energy source for pinocytosis by the oolemma. Although this is possible in some eggs, it seems unlikely for *Capitella* eggs since only the eggs of species I and II have this mitochondrial layer even though the level of endocytotic activity appears to be the same in the eggs of all the sibling species examined.

The significance of the amorphous electron-dense band in the cortical ooplasm of the eggs of species I, II, and IIIa is obscure. Some micrographs suggest that this layer is composed of fine filaments although this is uncommon in oocytes. Anderson (1969) described a prominent layer of filaments parallel to the oolemma in the

developing oocytes of the amphineurans *Mopalia mucosa* and *Chaetopleura apiculata* but did not speculate as to their possible significance. The amorphous substance observed in the eggs of *Capitella* might represent a less organized, non-filamentous form of microfilament similar to that described in the sperm duct epithelium of the ascidian *Ciona intestinalis* by Woollacott and Porter (1977). If the material in *Capitella* eggs indeed represents a microfilament reserve, the precise role of the putative organelles is problematical. They could serve a structural function, or be involved in morphogenetic movements, the fertilization reaction, or perhaps in the movement of mitochondria into the cortical ooplasm.

Wide variation in egg envelope morphology has been reported in different genera of polychaetes within the same family (Eckelbarger, in press) but never among closely related species of the same genus. This variation may be related to differences in the types of yolk precursors and metabolites being absorbed by the eggs during vitellogenesis or to the development of cross fertilization barriers. The morphological changes observed in the egg microvilli before and after ovulation in *Capitella* have not been previously described in polychaetes. This demonstrates that additional differentiation of the egg can occur following separation from the investing follicle cells which appear to be crucial to yolk synthesis.

Follicle cells are often associated with developing oocytes in polychaetes (Eckelbarger, in press) but extensive deposits of lipid and glycogen, as reported here in some *Capitella* ovaries, are uncommon. Eckelbarger (1979) reported some lipid and extensive deposits of glycogen in the follicle cells associated with the ovary in *Phragmatopoma lapidosa*. These deposits were believed to be utilized by the developing oocytes during vitellogenesis. Heacox and Schroeder (1981) observed lipid in the follicle cells associated with the gonial cell clusters in *Typosyllis pulchra* which they suggested might serve as nutrient material for the oocytes during development. In many polychaetes, the coelomic peritoneum often stores lipid and glycogen which are believed to serve a nutritive function during vitellogenesis, particularly in species undergoing extraovarian oogenesis (Eckelbarger, 1983). The ovarian follicle cells of *Capitella* are derived from the peritoneum, and the lipid and glycogen stores are believed to be destined for the developing oocytes (Eckelbarger and Grassle, 1982).

The differences reported here in the relative quantities of lipid and glycogen in the ovarian follicle cells of *Capitella* sibling species, presumably reflect the ultimate differences in types and quantities of yolk materials stored in the eggs. This in turn probably has a significant effect on embryogenesis and larval development particularly when egg size and subsequent developmental pattern (*i.e.*, planktotrophy versus lecithotrophy) vary so widely between the *Capitella* species under discussion. There are apparent differences in the general types of nutritive materials stored in the eggs of *Capitella* but unfortunately nothing is known of their chemical nature. It is tempting to use egg size as a unit of adult energy expenditure since it has been widely used in theoretical considerations of life history patterns (see Stearns, 1976) but it can be a misleading parameter which ignores organic composition (Turner and Lawrence, 1979). Indeed, in a study of the eggs of several invertebrate groups including polychaetes, Strathmann and Vedder (1977) reported that although organic matter per egg increases with egg diameter or volume, it is not directly proportional to egg volume because small eggs have more concentrated organic matter than larger eggs. It will be of interest to quantify the various organic components stored in the mature eggs of these *Capitella* sibling species (*i.e.*, proteid yolk, lipid, glycogen), to see how these materials might be utilized during embryogenesis and early larval development. This should help us better understand the developmental and ecological implications of the disparate distribution of nutrient material in the

follicle cells and eggs of *Capitella*. Laboratory studies of inbred strains of the lecitotrophic *Capitella* spp. I and II indicate that there is marked variation between lines in the capacity of the larvae to delay settlement in the absence of suitable substrate without suffering post-settling mortality (Grassle, unpub.). We expect these differences to be reflected in between-line differences in the amounts and/or types of nutritive materials incorporated into the eggs.

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